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Original Article

FERMENTATION EFFECTS ON CAFFEINE CONTENT AND CHEMICAL PARAMETERS OF KOMBUCHA COFFEE CASCARA

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ABSTRACT

Objective: The aim of this research is to examine the effect of varying fermentation on the caffeine content and chemical parameters (pH, the IC50 value, total phenolic and total flavonoid compounds) in kombucha robust coffee cascara (*Coffea canephora* Pierre ex A. Froehner).

Methods: The research was conducted by determining the caffeine content, pH, antioxidant activity, total phenolic and total flavonoid levels of the kombucha cascara robust coffee with variation concentration (1% and 3%) that was fermented with Symbiotic Culture of Bacteria and Yeast (SCOBY) over a period of 0, 3, 7, 14, and 21 d.

Results: The caffeine content varied from 42.99 mg to 23.36 mg in each serving. The pH values varied from 4.46 to 3.13. The IC50 value ranged from 134.48 µg/ml to 172.61 µg/ml. The total phenolic and total flavonoid compounds were 116.14±0.54 mg GAE/ml and 2.07±0.04 mg QE/ml, respectively.

Conclusion: The results showed that variations in fermentation affected the caffeine content, pH, the IC50 value, total phenolic and total flavonoid compounds of kombucha robust coffee cascara (*Coffea canephora* Pierre ex A. Froehner) as a functional drink.

Keywords: Fermentation, Kombucha, Cascara, Caffeine, Antioxidant

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INTRODUCTION

Coffee is classified into three types based on its cultivation location: Arabica coffee, Liberica coffee, and Robusta coffee. Robusta coffee can thrive in lowlands or coastal areas, boasting higher production rates than Arabica coffee. Despite its potential, coffee waste from these diverse regions remains largely untapped. Currently, its application is restricted, mainly limited to use as organic fertilizer. However, there is promising potential in converting coffee waste into cascara. This process is straightforward and uncomplicated, leveraging the abundance of potent antioxidants and essential nutrients found in cascara [1].

Plants, especially those rich in phenolic content and other bioactive components, can serve as valuable resources for the development of functional foods. This is primarily attributed to the antioxidant activity exhibited by their phytochemicals [2]. The increasing emphasis on natural antioxidants in food is propelled by their advantageous health and functional properties. The expectation is that advancements in understanding and utilizing natural antioxidants, particularly flavonoids and phenolic chemicals from plants, will contribute to overall safety. Phenolic compounds, which encompass various groups like phenols, flavonoids, tannins, and phenolic acids, represent a category of naturally occurring compounds with potential health benefits [3].

Kombucha, a fermented beverage originating from Manchuria, China, has transformed into a functional drink widely recognized for its medicinal purposes in East Asia [4]. Kombucha is renowned for its ability to provide antioxidants, showcase antibacterial properties, enhance physical resistance, reduce blood pressure, and maintain the equilibrium of probiotics in the digestive system. The starter culture employed in the fermentation of kombucha is referred to as SCOBY, an acronym for Symbiotic Culture of Bacteria and Yeast. This culture is a composite of both bacteria and yeast, with the bacterial group including *Acetobacter xylinum* and the yeast group comprising species from the *genera Brettanomyces, Zygisaccharomycetes, and Saccharomyces* [5].

The duration of fermentation significantly affects the reduction of caffeine levels, with an extended fermentation period allowing microorganisms to reach their optimal development stages. Within the SCOBY of kombucha, a diverse range of microorganisms generates various enzymes that play a crucial role in the fermentation process. The higher the concentration of microorganisms in kombucha, the greater the quantity of enzymes produced, contributing to the breakdown of caffeine content during fermentation [6]. Fermentation may have an effect on the phenolic compounds found in food products [7, 8]. Fermentation is a biotechnological process that enhances the nutritional value and sensory qualities of food. This involves the enzymatic transformation facilitated by microorganisms, converting conjugated phenolic forms into free phenolic forms [6].

Kombucha cascara robust coffee presents a promising avenue for a functional beverage. In response, a kombucha product was meticulously crafted using cascara from robust coffee, aiming to repurpose coffee skin waste. This study delves into the effect of varying fermentation durations on the caffeine content and several key chemical parameters, including pH, IC50 value, as well as total phenolic and total flavonoid compounds in kombucha derived from robust coffee cascara (*Coffea canephora Pierre ex A. Froehner*)

MATERIALS AND METHODS

Materials

Robust coffee (*Coffea canephora Pierre ex A. Froehner*) was sourced from Gajah Village, Berastagi District, Karo Regency, North Sumatra, at the ripe (red) level of maturity. Other materials, including caffeine, were procured from BPOM RI. Methanol, chloroform, sodium carbonate, aluminium chloride, DPPH (*1,1-Diphenyl-2-Pikrihidrazil*), *quercetin, folin-ciocalteu*, and *gallic acid* were obtained from Sigma-Aldrich. The study also incorporated white granulated sugar (*Gulaku*) and SCOBY (Symbiotic Culture of Bacteria and Yeast)

This study used analytical balance (Mettler Toledo), UV-Vis spectrophotometer (Shimidzu 1800), oven, pH meter, thermometer,

stopwatch and glassware (Iwaki Pyrex).

Methods

Preparation of cascara robust coffee

The robust coffee beans (*Coffea canephora Pierre ex A. Froehner*) were meticulously processed, beginning with sorting and cleaning under running water. After draining, the beans were left to dry in the shade for a day. Coffee peeling followed, involving the separation of the coffee skin from the beans. To produce cascara,, the coffee skins underwent drying process in an oven at 50±2 °C for five hours, followed by drying in a cabinet drier at 36 °C for seven days. The resulting dried cascara was thoroughly cleaned, ground into powder using a grinder, and then stored in a designated container for subsequent testing [9].

Preparation kombucha cascara robust coffee

The sugar solution was brought to a boil and mixed with cascara robust, previously prepared using hot water at a concentration of 2% (w/v). The mixture was homogenized by stirring for 5 min, then filtered to obtain the filtrate. The filtrate was cooled to approximately 30 °C and inoculated with 300 g of kombucha culture (SCOBY). Samples underwent fermentation for varying durations of 3, 7, 14, and 21 d at room temperature [10]. Do the same with ratio 1% and 3% of cascara and water.

Determination of caffeine content

The caffeine content was analyzed using UV-Vis spectrophotometry by measuring the absorption at the maximum wavelength of 273 nm. The recorded absorption values were then employed to determine the caffeine concentration, utilizing the regression equation derived from the standard calibration curve [11]. Caffeine levels in the sample can be calculated in the following way:

$$\text{Amount of caffeine (mg/ml)} = \text{concentration (µg/ml)} \times FP \times 10^{-3}$$

Where the regression equation y = ax+b was used and FP was dilution factor.

Antioxidant activity test

The DPPH method was utilized to evaluate the antioxidant activity of kombucha cascara robust coffee. Samples, subjected to five different fermentation durations (0, 3, 7, 14, and 21 d), were placed in volumetric flasks. Following this, 1 ml of 200 µg/ml DPPH was added to each flask, and the volume was adjusted to 5 ml with methanol. The mixture was homogenized, incubated for 30 min, and absorbance was measured using a spectrophotometer at the maximum absorption wavelength. This procedure was repeated three times for each fermentation duration to ensure the reliability and accuracy of the results [12]. The percentage of DPPH inhibition was calculated using the following formula:

$$\text{Percentage of DPPH inhibition} = \frac{\text{The absorbance of blank} - \text{the absorbance of sample}}{\text{The absorbance of blank}} \times 100\%$$

Where the absorbance of the blank refers to the absorbance of DPPH without added sample and the absorbance of the sample refers to the absorbance of the DPPH with the added sample.

Determination of total phenol content

The quantification of total phenol content utilized the Folin-Ciocalteu reagent, with gallic acid employed as a reference standard. UV-Vis spectrophotometry was employed to measure the absorption at a wavelength of 755 nm. The methodology is based on the formation of a blue complex compound resulting from the reaction between phenolic compounds and the Folin-Ciocalteu reagent under alkaline conditions. This intricate process was meticulously conducted with a triple repetition for each sample, ensuring a high degree of precision and reliability in the obtained results [13].

Determination of total flavonoid levels

The determination of total flavonoid levels was conducted using a colorimetric method with aluminum chloride reagent, and quercetin was employed as the standard. The quantification was performed through UV-Vis spectrophotometry at a wavelength of 442 nm. To ensure accuracy and reliability, measurements were repeated three times for each sample [13].

Evaluation of the pH value

The pH was measured using pH meter by dipping it into kombucha cascara robust coffee. The pH result will come out in monitor.

RESULTS

Caffeine content in Kombucha Cascara robust coffee

We conducted the fermentation process on cascara coffee beans for kombucha, employing a SCOBY (Symbiotic Culture of Bacteria and Yeast). The bacterial group included *Acetobacter xylinum*, while yeast microorganisms from the genera *Brettanomyces, Zygisaccharomycetes*, and *Saccharomyces* were also utilized.

The caffeine levels in kombucha cascara coffee beans are influenced by various factors, including the coffee type, geographical conditions of cultivation, degree of drying of the coffee skin, particle size of cascara coffee bean powder, brewing method, cascara coffee beans to water ratio during preparation, and post-harvest processing, particularly fermentation [6]. Table 1 presents the measured caffeine levels in kombucha cascara coffee beans.

Table 1: Caffeine levels in Kombucha Cascara robust coffee

Sample	Caffeine levels in kombucha cascara (mg/ml)±SD
No Fermentation	0.2150±0.00002
3th Fermentation day	0.1521±0.00005
7th Fermentation day	0.1260±0.00004
14th Fermentation day	0.1222±0.00008
21st Fermentation day	0.1168±0.00005

n=3

The results of measuring caffeine levels in kombucha cascara coffee beans, with variations in fermentation time, demonstrate that the duration of fermentation significantly influences the caffeine content in kombucha. This is evident when comparing the caffeine levels in kombucha without fermentation treatment to those with fermentation treatment. Notably, the longer the fermentation time, the lower the caffeine content observed in the sample. The research findings confirm the presence of caffeine in kombucha, although the amount found in cascara was lower than reported in the literature. The caffeine content in the cascara tea sample measured at 0.2150±0.00002 mg/ml in kombucha cascara, whereas the literature suggests cascara contains 226 mg/l of caffeine, equivalent to 0.226 mg/ml [14].

The caffeine content in kombucha decreases as fermentation progresses, reaching a plateau after the third day. The most significant reduction in caffeine occurs during the initial three days of fermentation, with the initial caffeine amount of 0.2150±0.00002 mg/ml decreasing to 0.1521±0.00005 mg/ml. However, beyond day 3th up to day 21st, the decline in caffeine content in kombucha stabilizes. This stabilization is attributed to the diminished optimal activity of yeast cells due to decreasing sugar content in kombucha and the formation of fermentation by-products, particularly organic acids, which contribute to a decline in kombucha pH. The comparison of the levels of caffeine in kombucha cascara with SNI can be viewed in the table 2.

Table 2 shown a comparison of the levels of caffeine in kombucha cascara coffee fruit research results that meet the quality standards set by SNI 01-7152-2006. The caffeine content in one glass of kombucha cascara coffee fruit for each serving (200 ml) is 42.99±0.00 mg; 30.42±0.01 mg; 25.21±0.01 mg; 24.44±0.02 mg and 23.36±0.01 mg. Based on table 2, it can be viewed that the caffeine content of kombucha cascara coffee fruit meets the maximum dose of caffeine consumption per day so that it meets SNI 01-7152-2006 because the caffeine content in kombucha in each serving does not exceed 50 mg.

The results of the analysis of caffeine levels in kombucha cascara coffee beans with various concentrations can be viewed in table 3.

Table 2: The comparison of the levels of caffeine in kombucha cascara robust coffee with SNI

Sample	Caffeine levels in kombucha cascara (mg/200 ml)±SD	SNI 01-7152-2006
No Fermentation	42.99±0.00	
3th Fermentation day	30.42±0.01	150 mg/d or
7th Fermentation day	25.21±0.01	50 mg/serving
14th Fermentation day	24.44±0.02	
21st Fermentation day	23.36±0.01	

n=3

Table 3: Caffeine levels in kombucha cascara robust coffee with various concentrations

Sample	Caffeine levels with various concentrations (mg/ml)±SD
1%	0.0392±0.00047
2%	0.0748±0.00004
3%	0.1164±0.00004

n=3

The results of measurements of caffeine levels in kombucha cascara robust coffee with various concentrations showed that the duration of fermentation affected the levels of caffeine contained in kombucha. This can be viewed in the results of measuring the caffeine content of 1% kombucha compared to 2% and 3% kombucha. The caffeine content in kombucha with a concentration of 1% (0.0392±0.00047 mg/ml) has a smaller amount than the caffeine content in kombucha with a concentration of 2% (0.0748±0.00004 mg/ml) and the caffeine content in kombucha with a concentration of 3% (0.1164±0.00004 mg/ml). Likewise, kombucha with a concentration of 2% has higher levels than kombucha with a concentration of 1% and lower than kombucha with a concentration of 3%. From the research results, kombucha with a concentration of 3% has the greatest caffeine content.

The results demonstrate a positive correlation between concentration and caffeine levels in the samples. This correlation aligns with studies that investigated caffeine levels across different ratios of ground coffee, revealing that lower concentrations of ground coffee in the sample were associated with reduced caffeine content. Additionally, other research has shown that both temperature and the quantity of samples brewed can influence the dissolved caffeine content in dry green tea products [15].

Antioxidant levels at variations in Kombucha Cascara robust coffee

Data on the results of calculating antioxidant levels at variations in kombucha cascara with fermentation time can be viewed in table 4.

Table 4: Result of antioxidant at variation kombucha cascara fermentation

Sample	IC_{50} (µg/ml)
No Fermentation	172.36
3th Fermentation day	146.59
7th Fermentation day	134.17
14th Fermentation day	140.23
21st Fermentation day	159.25
Quercetin	4.36

n=3

Antioxidant activity (IC_{50}) with varying concentrations of kombucha cascara coffee beans can be viewed in table 5.

Table 5: Results of antioxidant activity (IC_{50}) with varying concentrations of kombucha cascara robust coffee

Concentration	IC50 (µg/ml)
1 %	130.72 µg/ml
2 %	134.17 µg/ml
3 %	138.61 µg/ml

n=3

Total phenol content in kombucha cascara robust coffee

The test was carried out using a concentration of 500 µg/ml, determining the total phenol content in kombucha cascara robust coffee can be viewed in table 6.

Table 6: Total phenol content in Kombucha Cascara robust coffee

Sample	Total phenol content (mg GAE/ml) ±SD
No Fermentation	110.8693±0.8471
3th Fermentation day	113.3867±0.6769
4th Fermentation day	116.1411±0.5419
14th Fermentation day	100.7467±0.2663
21st Fermentation day	112.5219±0.1724

n=3

Based on the results of research conducted on kombucha cascara robust coffee, the phenol content values exhibited both increases and decreases. The observed rise in phenol values in kombucha from 0th day to day 7th fermentation is attributed to the initial phenol content it possesses. Phenolic compounds, influenced by factors such as the plant's growth environment and the availability of sufficient sunlight for photosynthesis, can increase during the fermentation process. This increase may be associated with tearubigin depolymerization that occurs during fermentation.

The phenol content decreased on 14th fermentation day, possibly due to the degradation of phenolic compounds such as catechin in tea. In contrast, the phenol content in kombucha cascara robust coffee increased on 21st. This increase is attributed to bacteria and yeast microbes metabolizing and producing flavonoid compounds through enzymatic reactions during fermentation, influencing the total phenol amount in kombucha [10]. The results of determining flavonoid levels in with varying concentrations of kombucha cascara robust coffee can be viewed in table 7.

Table 7: Total phenol content with varying concentrations of kombucha cascara robust coffee

Concentration	Total flavonoid levels (mg QE/ml)±SD
1 %	65.07±0.5518
2 %	116.1411±0.5419
3 %	125.53±0.4738

n=3

Total flavonoid contain in kombucha cascara robust coffee

The results of determining flavonoid levels in kombucha can be viewed in table 8.

Kombucha cascara robust coffee exhibited a sequential increase in levels during fermentation from no fermentation day to 7th fermentation day, with average values of 1.7717±0.0414, 1.8316±0.0916, and 2.0743±0.0454. Prolonged fermentation likely contributed to the elevation in total flavonoid levels, attributed to the antibacterial activity of lactic acid. This acid facilitates the production of enzymes that break down sugars, degrade complex phenolic compounds, and release phenolic compounds from the substrate, resulting in the formation of flavonoid compounds.

Table 8: Total flavonoids content in Kombucha Cascara robust coffee

Sample	Total flavonoid levels (mg QE/ml) ±SD
No Fermentation	1.7717±0.0414
3th Fermentation day	1.8316±0.0916
7th Fermentation day	2.0743±0.0454
14th Fermentation day	0.6935±0.0055
21th Fermentation day	1.8995±0.0076

n=3

In 14th fermentation, the total flavonoid content decreased to an average of 0.6935±0.0055, possibly due to increased organic acids resulting from yeast and bacteria activity in kombucha fermentation. However, during 21st d fermentation, flavonoid levels rebounded, reaching an average of 1.8995±0.0076. This increase is attributed to lactic acid bacterial fermentation, where enzymes break down sugars degrade complex phenolic compounds, leading to an increase in the phenolic group formation and the development of flavonoid compounds.

The results of determining flavonoid levels with varying concentrations of kombucha cascara robust coffee can be viewed in table 9.

Table 9: Total flavonoids content with varying concentrations of kombucha cascara robust coffee

Concentration	Total flavonoid levels (mg QE/ml)±SD
1 %	0.0014±0.0263
2 %	2.0743±0.0454
3 %	7.9983±0.0135

n=3

pH value

In the process of kombucha cascara coffee berry fermentation, there was a significant decrease in pH along with the length of time the kombucha fermented. The results of measuring the pH of kombucha cascara robust coffee can be viewed in table 10.

Table 10: Measurements of the pH of Kombucha Cascara robust coffee

Sample	Mean pH
No Fermentation	4.46
3th Fermentation day	3.35
7th Fermentation day	3.30
14th Fermentation day	3.25
21st Fermentation day	3.13

n=3

Throughout the fermentation process of kombucha cascara robust coffee, a significant reduction in pH occurred over time. This decline is attributed to the accumulation of organic acids and an increase in protons (H+) resulting from bacterial metabolism. The organic acids generated include acetic acid, glucuronic acid, and gluconic acid [16]. Increasing the acid content in the sample results in a decrease in pH, consistent with the observation that pH tends to rise with prolonged fermentation. Moreover, samples with lower caffeine content exhibit higher acidity levels (pH) [17].

Analysis statistic

The study demonstrates a significant effect of fermentation duration on the levels of antioxidants, caffeine, phenols, and flavonoids in kombucha cascara robusta coffee cherry (*Coffea canephora Pierre ex A. Froehner*) with a probability of 0.00 (*P<0.05). In 200 ml servings, caffeine content varied with fermentation time: 42.99±0.00 mg (lowest at 21 d). Caffeine levels were also influenced by cascara concentration, with 1%, 2%, and 3% concentrations resulting in 0.0392±0.00047 mg/ml, 0.0748±0.00004 mg/ml and 0.1164±0.00004 mg/ml respectively.

The study identified antioxidant activity in kombucha cascara coffee beans at various fermentation times (0, 3, 7, 14, 21) d. The IC50 values were 172.36 µg/ml, 146.59 µg/ml, 134.17 µg/ml, 140.23 µg/ml, and 159.25 µg/ml, respectively. Additionally, water cascara extract and quercetin had IC50 values of 32.00 µg/ml and 4.36 µg/ml. The research established a significant effect of fermentation duration on antioxidant activity in kombucha cascara coffee fruit, with the optimal IC50 value observed at the 7th fermentation time.

There were total phenols and total flavonoids in dry coffee fruit skin kombucha with variations in no fermentation time; 3th; 7th; 14th; 21st, respectively, namely 110.8693 mg GAE/ml; 113.3867 mg GAE/ml; 116.1411 mg GAE/ml; 100.7476 mg GAE/ml; 112.5219 mg GAE/ml;

1.7717 mg QE/ml; 1.8316 mg QE/ml; 2.0743 mg QE/ml; 0.6935 mg QE/ml; 1.8995 mg QE/ml.

The pH value before fermentation was 4.46 and after it was fermented, the pH value for kombucha was 3.35; 3.30; 3.25 and 3.13.

DISCUSSION

Caffeine levels in Kombucha Cascara robust coffee

The research confirms the presence of caffeine in kombucha, as evidenced by analysis at a wavelength of 273 nm during various fermentation times. However, the caffeine content in kombucha cascara was lower than reported in the literature, with only 0.2150±0.00002 mg/ml, compared to the literature's reported 226 mg/l or the equivalent of 0.226 mg/ml in cascara tea [20]. Several factors may contribute to the observed differences in caffeine content, including the type of coffee and geographical conditions of cultivation, roasting level, particle size of cascara powder, brewing method, cascara-to-water ratio, post-harvest processing (fermentation), cleanliness of the container used, and the potential for fermentation during the cascara drying period. All these factors can influence the final caffeine levels [5].

The caffeine levels in kombucha cascara robust coffee varied with fermentation time, clearly indicating the effect of fermentation duration on caffeine content. A comparison between kombucha without fermentation treatment and those subjected to fermentation revealed that as fermentation time increased, the caffeine content in the sample decreased. In kombucha, microbes, particularly those in SCOBY, produce enzymes such as caffeine demethylase. These enzymes break down complex caffeine compounds during fermentation.

The abundance of microorganisms correlates with the increased number of enzymes involved, leading to the conversion of caffeine into theobromine, theophylline, paraxanthine, 3-methylxanthine, 7-methylxanthine, 1-methylxanthine, and xanthine. The resulting xanthine compounds will be converted into intermediate metabolic products such as uric acid, allantoin, allantoate, ureidoglycolate, glyoxylate, CO2, and NH3 [16].

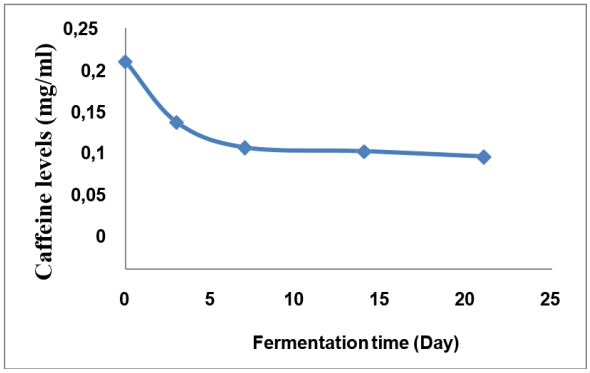

Fig. 1: Curve of decreasing caffeine content over fermentation time

Fig. 1 demonstrates a decline in caffeine levels during the fermentation of kombucha, stabilizing after day 3. The most substantial reduction occurred within the initial three days, with the initial caffeine content of 0.2150±0.00002 mg/ml decreasing to 0.1521±0.00005 mg/ml. Optimal microbial activity during this period, facilitated by high sugar content, a suitable fermentation temperature, and minimal formation of fermentation by-products, contributed to this decline. However, from 3th d to 21st d, the reduction in caffeine became stagnant. Yeast cells were no longer functioning optimally due to lower sugar content and the formation of organic acids began to lower the pH of the kombucha.

During the fermentation process, the microbial population experiences a decline due to bacterial inactivity. This inactivity arises from a depletion of essential nutrients utilized by the microbes to produce acid metabolites. As a consequence, the microbes either undergo a reduction in number, signifying the completion of the fermentation process, or they may perish. The decrease in the overall microbe count can also be attributed to the conversion of aliphatic acids into carboxylic acid esters, resulting in fermentation defects characterized by an undesirable rotten taste [18].

In addition to the limited availability of nutrients, the elevated levels of alcohol produced during the fermentation process serve as another significant factor contributing to microbial death, thereby impeding the optimal progression of fermentation. This aligns with the assertion that an extended fermentation period leads to a reduction in microbial count, entering a death phase characterized by the accumulation of alcohol and a depletion of substrate. The increased presence of alcohol during fermentation can induce yeast cell death, referred to as autotoxin, further exacerbating the challenges faced in achieving an efficient fermentation process [5].

Antioxidant levels at variations in Kombucha Cascara robust coffee

The results showed that there was an increase and decrease in the antioxidant activity of kombucha cascara during fermentation due to changes in the pH value, where the longer the fermentation, the lower the pH value of kombucha. This can have an effect on the occurrence of damage to phenols which act as antioxidants. The instability of antioxidants in acidic conditions is due to the presence of compounds that have active groups, namely hydroxyl groups, which can act as free anti-radicals by donating unpaired electrons to radical compounds so that free radicals become stable. The antioxidant activity of kombucha cascara coffee beans with a fermentation time of 0 d tends to be lower than the antioxidant activity of kombucha cascara coffee beans that have undergone a fermentation process. This was because the fermentation process had not occurred on that day, the microorganisms contained in the solution had not undergone a metabolic process, so it can be said that the antioxidant activity contained in kombucha cascara coffee cherries at no fermentation day came from the phenolic compounds contained in cascara coffee fruit [10].

The IC50 value of kombucha cascara coffee fruit as shown in table 5 ranges from 134.17 µg/ml -172.36 µg/ml. It can be categorized as moderate antioxidants, whereas no fermentation, 14th fermentation, and 21st fermentation day are categorized as weak antioxidants. The lowest antioxidant activity was shown by the control, which was 0 d of fermentation with an IC50 value of 172.36 µg/ml, and the highest antioxidant activity was shown at 7th fermentation day with an IC50 value of 134.17 µg/ml.

Based on the results of the research that has been done, it shown an increase and decrease in the antioxidant activity of kombucha cascara during the fermentation process. It is caused by changes of the pH value, where the longer the fermentation process, the pH value of kombucha will decrease. This can have an effect on the occurrence of damage to phenols which act as antioxidants. The instability of antioxidants in acidic conditions is due to the presence of compounds that have active groups, namely hydroxyl groups which can act as free anti-radicals, by donating unpaired electrons to radical compounds so that free radicals become stable [19, 20].

When the fermentation took place on the 14th to the 21st d of fermentation, there was a decrease in antioxidant activity. It can be caused because during the fermentation process, there is an increase in the amount of organic acids due to the activity of yeast and bacteria that are in kombucha. The acidic atmosphere causes the phenolic compounds to become more stable and it is difficult to release protons which can bind to DPPH so that their antioxidant activity decreases [21]. When compared the IC50 value of the quercetin comparator with the IC50 value of kombucha cascara coffee fruit obtained, it is known that the IC_{50} value of quercetin is higher. This indicates that the antioxidant properties of the bioactive compounds contained in kombucha cascara coffee beans are weaker when compared to quercetin. The smaller the IC50 value, the greater the antioxidant activity of the tested material [11].

The results of measuring the IC50 value of kombucha cascara coffee beans with various concentrations shown that the duration of fermentation affected the antioxidant activity contained in kombucha. This can be viewed in the results of measuring the caffeine content of 1% kombucha compared to 2% and 3% kombucha. The IC50 value in kombucha with a concentration of 1% (130.72 µg/ml) is smaller than the IC50 value in kombucha with a concentration of 2% (134.17 µg/ml) and 3% concentration (138.61 µg/ml). Likewise, kombucha with a concentration of 2% has a greater IC50 value than kombucha with a concentration of 1% and lower than kombucha with a concentration of 3%. From the research results, kombucha with a concentration of 1% have the lowest IC50 value. Where the smaller the IC50 value, the greater the antioxidant activity.

Total phenol and flavonoids level

Based on the results of this research, the phenol content values increased and decreased. The increase in phenol value in kombucha at 0th to 7th fermentation day is influenced by the phenol content it contains, where phenolic compounds are influenced by the place of growth and the availability of sufficient sunlight for photosynthesis. Phenolic compounds can increase due to the fermentation process because during this process tearubigin depolymerization may occur. This is because during the fermentation process, kombucha tea has four isomers of epicatechin, including epihalocatechin gallate, epicatechin gallate, epigalocatechin, and eoikatechin. These isomers can undergo a biotransformation process by enzymes produced from the metabolism of microorganisms. The process of biotransformation of epigalocatechin gallate into epigalocatechin and epicatechin gallate and the release of catechin from microorganism cells that are sensitive to acid, so that this process is thought to increase polyphenols during fermentation [21, 22].

The total phenol content decreased on 14th fermentation day, which could be due to the degradation of phenolic compounds, one of which is catechin contained in tea brewing at the start of fermentation. Catechins that are lost and further polymerized into molecules with a higher weight lead to the detection of lower polyphenol content [23]. The fermentation process causes the polyphenol content to decrease due to an oxidation reaction [23, 24]. The decrease in phenolic levels is related to the decrease in the number of microbial cells because reducing sugars as an energy source for microbes also decrease. The decrease in phenol levels occurred due to the concentration of sugar used [23], which caused the absorbance in 14th fermentation to decrease. The phenol content of kombucha cascara robust coffee in 21st d is increase. The increase of phenolic compounds during fermentation is thought to be due to bacteria and yeast microbes, which can metabolize to produce flavonoid compounds through enzymatic reactions, thus affecting the total amount of phenols in kombucha [10].

Kombucha cascara robust coffee experienced an increase in levels from 0th to 7th fermentation day with a sequential average of 1.7717±0.0414; 1.8316±0.0916; 2.0743±0.0454. The longer fermentation can cause an increase in the value of total flavonoid levels; this can occur due to the antibacterial activity of lactic acid, which is able to produce enzymes to break down sugars and degrade complex phenolic compounds and release phenolic compounds from the substrate, this causes the formation of flavonoid compounds. Lactic acid bacteria found in SCOBY, such as Lactobacillus plantarum and Lactobacillus acidophilus, can degrade polyphenolic

compounds, which states that during the fermentation process, flavonoid compounds can experience degradation or are formed from the degredation of polyphenolic compounds [5].

In 14th fermentation day the total flavonoid content decreased with an average level 0.6935±0.0055 then the average value of flavonoid levels rise again in 21st fermentation day with an average level of 1.8995±0.0076. The decrease in flavonoid levels can be caused during the process. In fermentation, there is an increase in the amount of organic acids due to the activity of yeast and bacteria contained in kombucha. An acidic atmosphere causes phenolic compounds to become more stable and difficult to release protons which are related to antioxidant activity [21]. The cause of the decrease in flavonoid values can be influenced by the flavonoid content itself, where these flavonoid compounds are influenced by the place of growth and the availability of sufficient sunlight for photosynthesis [22]. In 21st fermentation day the total flavonoid levels again increased because during lactic acid bacterial fermentation will produce enzymes that can break down sugars and degrade complex phenolic compounds as well as release phenolic compounds from the substrate so that the phenolic group increases and forms flavonoid compounds. During the fermentation process, microbes will convert phenolic compounds into simple compounds, one of which is flavonoids.

CONCLUSION

The results showed that variations in fermentation time affected the caffeine content, pH, the IC50 value, total phenolic and total flavonoid compounds of kombucha robust coffee cascara (*Coffea canephora Pierre ex A. Froehner*) as a functional drink with a probability of 0.00 (*P<0.05).

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AUTHORS CONTRIBUTIONS

Conceptualization: LRN, YMP, JMK, MATP, VKC, TRS; table work: MATP, VKC, TRS; Supervision: LRN, YMP, JMK; Revision: LRN, MATP, VKC, TRS; Writing and Editing: LRN; JMK; MATP; Proofreading: LRN, YMP, JMK.

CONFLICT OF INTERESTS

The authors have no conflicts of interest regarding this research.

REFERENCES

1. Murlida E, Noviasari S, Nilda C, Rohaya S, Rahmi F, Muzaifa M. Chemical characteristics of cascara tea from several varieties of coffee in aceh province. IOP Conf Ser.: Earth Environ Sci. 2021;667(1). doi: 10.1088/1755-1315/667/1/012078.
2. Jimenez Lopez J, Ruiz Medina A, Ortega Barrales P, Llorent Martinez EJ. Phytochemical profile and antioxidant activity of caper berries (Capparis spinosa L.): evaluation of the influence of the fermentation process. Food Chem. 2018 Jun;250:54-9. doi: 10.1016/j.foodchem.2018.01.010, PMID 29412927.
3. Chung IM, Seo SH, Ahn JK, Kim SH. Effect of processing, fermentation, and aging treatment to content and profile of phenolic compounds in soybean seed, soy curd and soy paste. Food Chem. 2011;127(3):960-7. doi: 10.1016/j.foodchem.2011.01.065.
4. Putri Rahayu Y, Sari Lubis M, Nuraida. Pkm manufacture probiotic fermentation products pickled cucumber as functional food for health in building innovation in the era of industrial revolution 4.0 and society 5.0 to teacher sma plus Taruna field actor. Pros Semin Has Pengabdi. 2022;1:331-41.
5. Khaerah A, Akbar F. The antioxidant activity of Kombucha from several different varieties of tea. Pros Semin LP. 2019;2M UNM:472-6.
6. Kemurnian P, Dan E, Pada P, Antioksidan A, Brawijaya U, Teknik F. Skripsi teknik kimia; 2017.
7. Frias J, Miranda M, Doblado R, Vidalvalverde C. Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of L. var. Multolupa. Food Chem. 2005;92(2):211-20. doi: 10.1016/j.foodchem.2004.06.049.
8. Septembre Malaterre A, Remize F, Poucheret PP. Fruits and vegetables, as a source of nutritional compounds and phytochemicals: changes in bioactive compounds during lactic fermentation. Food Res Int. 2018 Feb;104:86-99. doi: 10.1016/j.foodres.2017.09.031, PMID 29433787.
9. Irwinsyah AD, Assa JR, Oessoe YY. Antioxidant activity analysis with Dpph method as well as Koya arabica coffee acceptance rate. TjyybjbAcCn. 2019;3(2):58-66.
10. Nummer BA. Kombucha brewing under the food and Drug Administration model food code: risk analysis and processing guidance. J Environ Health. 2013 Nov;76(4):8-11. PMID 24341155.
11. Isnindar SW, Widyarini S, Yuswanto. Analysis of the caffeine content in melted coffee bean extract from Yogyakarta special territory farms using UV-vis spectrophotometry. Pharmacon. 2016;5(2):838-41.
12. Fitrotunnisa Q, Arsianti A, Tejaputri NA, Qorina F. Antioxidative activity and phytochemistry profile of hibiscus sabdariffa herb extracts. Int J App Pharm. 2019;11Special Issue 6:29-32. doi: 10.22159/ijap.2019.v11s6.33532.
13. Christiani Dwiputri M, Lauda Feroniasanti YM. Effect of fermentation to total titrable acids, flavonoid and antioxidant activity of butterfly pea kombucha. J Phys.: Conf Ser. 2019;1241(1). doi: 10.1088/1742-6596/1241/1/012014.
14. Nurhayati N, Yuwanti S, Urbahillah A. Physicochemical and sensory characteristics of kombucha cascara (kulit kopi Ranum). JTIP. 2020;31(1):38-49. doi: 10.6066/jtip.2020.31.1.38.
15. Annuryanti F, Zahroh M, Purwanto DA. Influence of temperature and injection quantity on the level of dissolved caffeine in dry green tea products by KCKT method. J Farm dan Ilmu Kefarmasian Indones. 2019 Mar 21;5(1):30-5.
16. Lestari D, Kadirman K, Patang P. Subtitulation of falcon seed powder and arabica coffee powder in the manufacture of coffee powder. J Pendidik Teknol Pertan. 2018;3(1):15.
17. Aini AF, Saripah MR, Hariyadi T. Effects of filling and fermentation time on caffeine level, pH value, and ethanol value of arabica coffee bean fermenting results. Pros 12th Ind Res Work Natl Semin. 2021;12:145-50.
18. Katalinić V, Milos M, Modun D, Music I, Boban M. Antioxidant effectiveness of selected wines in comparison with (+)-catechin. Food Chem. 2004;86(4):593-600. doi: 10.1016/j.foodchem.2003.10.007.
19. Di Lorenzo C, Badea M, Colombo F, Orgiu F, Frigerio G, Pastor RF. Antioxidant activity of wine assessed by different *in vitro* methods. BIO Web Conf. 2017;9:04008. doi: 10.1051/bioconf/20170904008.
20. Riza Marjoni M, Devi Novita A. Total phenol content and antioxidant activity of kersen leaf water extract (Muntingia calabura L.) total content of fenol and antioxidant activity of the aqueous extract of cherry leaf (Muntingia calabura L.). J Kedokt Yars. 2015;23(3):187-96. doi: 10.33476/jky.v23i3.232.
21. Nurhayati N, Belgis M, Yuwanti S, Sari P, Yuliany NN. Kombucha cascara technology for coffee groups "TANI MAJU" Sukorejo Village, Bondowoso District. J Teknol Has Pertan. 2018:122-5.
22. Primurdia E, Kusnadi J. Antioxidant activity of the probiotic drink of sar I. kurma (Phoenix dactilyfera L.) with l. plantarum and l. casei isolates antioxidant. J Pangan Dan Agroindustri. 2014;2(3):98-109.
23. Rosyada FF, Agustina E, Faizah H. The effect of fermentation on the characteristics and antioxidant activity of wuluh starfruit leaf kombucha tea (Avverhoa bilimbi Linn.). Indo J Chem Res. 2023;11(1):29-36. doi: 10.30598/ijcr.2023.10-agu.
24. Ardheniati M, Andriani MA, Amanto BS. Fermentation kinetics in kombucha tea with tea kind variation based on its processing. J Nat Prod Biochem. 2009;7(1):48-55. doi: 10.13057/biofar/f070106.